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# EFFECTS OF HYPOXIC STRESS ON SURVIVAL TIMES OF MICE INFECTED WITH EITHER 'STAPHYLOCOCCUS AUREUS' OR 'KLEBSIELLA PNEUMONIAE'

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COMPARATIVE STUDIES OF THE EFFECT OF HYPOXIC STRESS ON INFECTION WITH GRAM POSITIVE OR GRAM NEGATIVE BACTERIA WERE CONDUCTED. SURVIVAL TIMES OF MICE INFECTED WITH EITHER S. AUREUS OR K. PNEUMONIAE WERE SIGNIFICANTLY EXTENDED BY TREATMENT WITH 10% OXYGEN BEGUN IMMEDIATELY POST INFECTION. DELAYED TREATMENT WAS INEFFECTIVE. HYPOXIA INDUCED BY 0.6 ATM. WAS DETRIMENTAL IN TREATMENT OF INFECTED MICE. THIS LATTER TREATMENT HAD NO EFFECT ON BACTERIAL COUNTS IN ORGANS OF INFECTED MICE. TREATMENT WITH 10% OXYGEN DID, HOWEVER, INCREASE NUMBERS OF S. AUREUS (OVER)

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IN LUNGS AND LIVERS OF INFECTED MICE. NO EFFECT OF THIS TREATMENT ON BACTERIAL COUNTS WAS SEEN IN MICE CHALLENGED WITH K. PNEUMONIAE. MICE WITH DIFFUSE TYPE HAEMOGLOBIN WERE FOUND TO BE MORE RESISTANT TO BOTH INFECTIONS THAN ANIMALS WITH THE SINGLE TYPE HAEMOGLOBIN. THUS, UNDER SOME CIRCUMSTANCES, HYPOXIA CAN PROLONG SURVIVAL TIMES OF MICE INFECTED WITH S. AUREUS OR K. PNEUMONIAE, APPARENTLY THROUGH EFFECTS ON HOST METABOLISM.

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# EFFECTS OF HYPOXIC STRESS ON SURVIVAL TIMES OF MICE INFECTED WITH EITHER *STAPHYLOCOCCUS AUREUS* OR *KLEBSIELLA PNEUMONIAE*

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## INTRODUCTION

The host-microbial relationship may be modified by hypoxia arising naturally or under laboratory circumstances. Particular attention has been directed toward effects of hyperoxia and hypoxia on established infections (Schmidt, 1969; Schmidt and Ball, 1970; Angrick *et al.*, 1971). Since tissue hypoxia may be a major result of circulatory impairment associated with severe bacterial infection (Siegel *et al.*, 1967; Duff *et al.*, 1969; Rhoden *et al.*, 1969), attempts have been made to alleviate this deficit through treatment with supplementary oxygenation. (Blair *et al.*, 1964; Barnwell *et al.*, 1966). With aerobic microorganisms this approach is often ineffective.

In contrast, survival times may be prolonged if the host animal is subjected to hypoxic stress. For example, mice infected with *Staphylococcus aureus* have been reported to demonstrate significantly prolonged survival times following treatment with hypoxic stress initiated by 0.6 atm. or 10% oxygen (Barnwell *et al.*, 1966). This treatment should contribute to tissue hypoxia originating from infection, so the beneficial effect derived must be a result of alteration of metabolic activities which are of more immediate importance to survival.

Few attempts have been made to analyze mechanisms by which hypoxic stress may affect the infection process. Investigations of this type can provide an understanding of some of the effects of hypoxic stress as well as the importance of certain metabolic events associated with infection. The following study was undertaken to determine the effects of hypoxic stress on survival times of mice infected with *S. aureus* or *Klebsiella pneumoniae*.

## MATERIALS AND METHODS

**Infection :** *S. aureus* and *K. pneumoniae* from 18 hour Brain Heart Infusion broth cultures were washed and resuspended in saline at a concentration of $10^9$ viable cells per ml. Random-bred female Swiss mice ten weeks of age (25-30 gm) were inoculated intraperitoneally with 1 c.mm of the bacterial suspension. Such large doses are not lethal when the cells are killed before inoculation and host responses to the live cell injections resemble those associated with more natural infections.

**Treatment :** Immediately following inoculation, mice were either exposed to 20%

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oxygen (room air) or to 10% oxygen in a 4-liter chamber through which oxygen-nitrogen from a tank of premixed gas was fed (Fig. 1). Flow rate was adjusted to approximately 0.5 kg/cm/min and temperature inside the bottle was the same as room temperature.

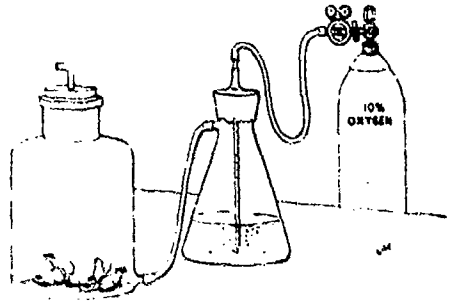


Fig. 1—Premixed 10% oxygen was passed through a water trap into 4 liter bottles in which mice were contained.

Simulated altitude was achieved in a 6-man cylindrical altitude chamber 12 feet in length and 8 feet in diameter. The ascending rate of the chamber was 2000 feet per minute until it reached 450 mmHg (approximately 13,500 feet) or 0.6 standard atmosphere. Ventilation of the chamber was continued through the course of the experiments. Control mice were held immediately outside the chamber. Temperatures inside and outside the chamber were identical.

Survival time from infection until death was recorded for mice in each of the experimental groups and significance of the mean was determined for the first 50% of the mice to die.

**Bacteriologic examination:** Organs removed from infected mice were homogenized in sterile saline, serially diluted, and plated on Brain Heart Infusion plates. Cultures were quantitated after 18 hours.

**Haemoglobin analysis:** Blood was obtained from each mouse by retroorbital bleeding and allowed to clot. Haemoglobin types were determined by vertical starch gel electrophoresis using minor modifications of the Smithies procedure (Blackwell and Huang, 1965).

**Statistical analysis:** Significance of differences in survival times was determined by a 't' test.

## RESULTS

### Mouse survival studies

Treatment with 10% oxygen immediately after inoculation with *S. aureus* or *K. pneumoniae* was a significant factor in extending survival times when compared to those of control mice breathing room air (Table 1). In contrast, hypoxic stress initiated by reduced pressure (0.6 atm.) was not only ineffective in extending survival times of mice infected with either organism but actually shortened survival times. When treatment with 10% oxygen was delayed 150 minutes after inoculation of mice with *S. aureus* or *K. pneumoniae* no significant alteration of survival time was obtained (Table 2). Experiments with the Smith strain of *S. aureus* gave results identical to those obtained with the Giorgio strain.

### Effects of hypoxia on organ bacterial counts

Quantitation of *S. aureus* and *K. pneumoniae* in host tissue after inoculation was conducted in order to detect possible antibacterial activity of hypoxic stress. Comparison of plate counts demonstrated no difference in numbers of viable organisms in lungs or livers of mice infected with *K. pneumoniae* and exposed to 10% oxygen or normal room air (Table 3). Pulmonary and hepatic tissue of mice exposed to the same conditions and infected with *S. aureus*, however, was more heavily invaded following hypoxic treatment. Treatment with 0.6 atm. had no appreciable effect on bacterial counts.

Table 1

MST[50]* of infected mice** subjected to treatment with reduced concentration and/or pressure of oxygen.

| *Staphylococcus aureus* | | | | *Klebsiella pneumoniae* | | | |
|---|---|---|---|---|---|---|---|
| 1 atm. 20% $O_2$ | 0.6 atm. 20% $O_2$ | 1 atm. 10% $O_2$ | 0.6 atm. 10% $O_2$ | 1 atm. 20% $O_2$ | 0.6 atm. 20% $O_2$ | 1 atm. 10% $O_2$ | 0.6 atm. 10% $O_2$ |
| 249.3±9.9 | 233.4±16.9*** | 316±26.5 | 279.1±31.1*** | 230.9±24.3 | 215.6±18.3 | 257.8±30.8 | 267.5±33.0 |
| 1 atm. 20% $O_2$ | 1 atm. 10% $O_2$ | 0.6 atm. 20% $O_2$ | 0.6 atm. 10% $O_2$ | 1 atm. 20% $O_2$ | 1 atm. 10% $O_2$ | 0.6 atm. 20% $O_2$ | 0.6 atm. 10% $O_2$ |
| 249.3±9.9 | 316.0±26.5*** | 233.4±16.9 | 279.1±31.1*** | 230.9±24.3 | 257.8±30.8*** | 215.6±18.3 | 267.5±33.0 |
| 1 atm. 20% $O_2$ | 0.6 atm. 10% $O_2$ | 1 atm. 10% $O_2$ | 0.6 atm. 10% $O_2$ | 1 atm. 20% $O_2$ | 0.6 atm. 10% $O_2$ | 1 atm. 10% $O_2$ | 0.6 atm. 20% $O_2$ |
| 249.3±9.9 | 279.1±31.1*** | 316.0 ± 26.5 | 233.4±16.9*** | 230.9±24.3 | 267.5±33.0*** | 257.8±30.8 | 215.6±18.3*** |

* Mean survival time (in minutes) for first 50% of the mice to die. Expressed as mean ± standard deviation.
** Each group consists of 20 mice. Comparison of MST[50]'s involved 10 mice from each group.
*** Significantly deviated from value to which compared ($p \leq 0.05$).

Table 2

Effect of delayed treatment* with 10% $O_2$ on survival times of mice infected with *Klebsiella pneumoniae* or *Staphylococcus aureus*.

| | *S. aureus* | | *K. pneumoniae* | |
|---|---|---|---|---|
| MST[50]** | 20% $O_2$ | 10% $O_2$ | 20% $O_2$ | 10% $O_2$ |
| | 218.3±21.1 | 220.2±27.2 | 274.8±65.7 | 272.0±39.2 |
| No. Mice | 20 | | 19 | |

*Treatment with hypoxic stress begun 150 minutes following inoculation of mice with $10^9$ viable organisms.
**Mean survival time (in minutes) for first 50% of the mice to die. Expressed as mean±standard deviation.

Table 3

Bacterial invasion* of livers and lungs of mice exposed to hypoxic stress.

| | *K. pneumoniae* | | *S. aureus* | |
|---|---|---|---|---|
| | 20% $O_2$ | 10% $O_2$ | 20% $O_2$ | 10% $O_2$ |
| Liver | $1.4\times10^8$ | $1.2\times10^8$ | $7.0\times10^7$ | $1.1\times10^8$ |
| Lung | $2.4\times10^8$ | $3.7\times10^8$ | $1.5\times10^6$ | $9.0\times10^6$ |

*Organisms/ml homogenate obtained from average of viable counts of livers or lungs from three mice. Similar results were obtained in repeated experiments.

**Relationship of haemoglobin type to susceptibility to infection**

The electrophoretic patterns of haemoglobins for all mice tested fell into two principal groups, single and diffuse, as described by previous workers (Schmidt and Ball, 1970); the mice with the single haemoglobin pattern constituted approximately three-fourths of those tested. Both major haemoglobin groups could be subdivided electrophoretically, (Fig. 2), but, as a result of preliminary studies, were only regarded as two groups on the basis of survival. Untreated mice with the diffuse haemoglobin pattern were more resistant to infection with *S. aureus* or *K. pneumoniae* than those with the single pattern (Table 4). Mice with either single or diffuse haemoglobin types responded to the beneficial effects of 10% oxygen treatment after infection with *S. aureus*.

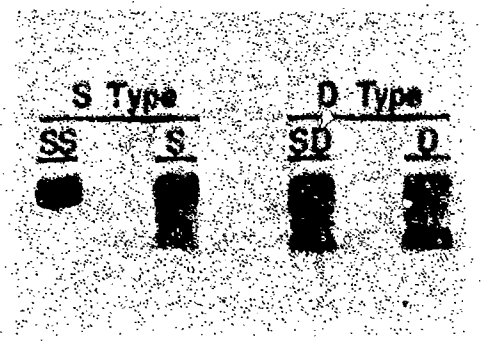


Fig. 2—Electrophoretic patterns of mouse haemoglobin.

## DISCUSSION

Although tissue hypoxia may be an ultimate result of infection, hypoxic stress can, if begun before infection is well established, affect lethal events which normally occur later during the infection sequence. Altitude-

Table 4

Relationship of haemoglobin type and survival time* following bacterial infection.

| | *K. pneumoniae* | | *S. aureus* | |
|---|---|---|---|---|
| | Single Type | Diffuse Type | Single Type | Diffuse Type |
| Survival Time | 254.4±54.9 | 299.6±60.0** | 221.6±10.4 | 248.7±17.5** |
| No. Samples | 51 | | 45 | |

*Mean survival time (in minutes) ± standard deviation.
**Significantly different from single type haemoglobin survival value ($p \leq 0.05$).

dependent regulation of enzyme activity has been previously reported (Berry, 1971) and could influence host survival by increasing pools of certain essential enzymes. The relatively short time span of the experiments in our study makes actual enzyme synthesis unlikely. Activation of certain pre-existing enzymes and alteration of metabolic or physiologic processes normally present would be a more probable occurrence.

The fact that 10% oxygen is equivalent to the hypoxic stress of a 5000 ft greater altitude than 0.6 atm. may be responsible for the difference observed in effect on survival times. Previous investigations with *S. aureus* (Barnwell *et al.*, 1966) demonstrated that prolonged host survival times could be obtained with either degree of hypoxic stress and that their effects were additive. In an attempt to explain this discrepancy, the Smith strain of *S. aureus* used in these investigations and the Giorgio strain reported here were compared in a repeated set of survival experiments using 10% oxygen and 0.6 atm. as treatments. Identical results supporting our previous observations were obtained with both strains of *S. aureus*. This leaves the possibility that differences in mouse strains used could account for variation in results obtained.

Specific adaptation to hypoxic stress could prepare the host for infection-induced hypoxia (Oski *et al.*, 1970; Finch and Lenfant, 1972). For this reason factors which regulate haemoglobin affinity for oxygen were considered. A protective role for haemoglobin oxygen affinity during infection was indicated by the correlation between haemoglobin type and survival time of untreated mice following bacterial challenge. Physiologic studies of the two haemoglobin types should be considered to determine if their oxygen affinities are significantly different. A change of one amino acid in the molecule could affect the allosteric response of the haemoglobin molecule to oxygen loading and other ligands (Finch and Lenfant, 1972). Other traits genetically associated with haemoglobin could also be responsible for the enhanced resistance to infection. Data in preparation, however, will show that at least part of the effect of hypoxic treatment in infected animals is through partial retardation of 2, 3-diphosphoglycerate depletion. This depletion is known to occur during septic shock (Oski *et al.*, 1970), and its retardation would promote delivery of oxygen to host tissues (Eaton *et al.*, 1970; Baumann *et al.*, 1971).

Enhanced phagocytic activity has been suggested as a possible explanation for the beneficial effects of hypoxic treatment on infection (Schmidt and Ball, 1970). Alterations of bacterial numbers, however, do not seem to account for effects on survival times reported here. Increased numbers of staphylococci in tissues of mice treated with 10% oxygen may be more important in prolonged, sublethal infections. This could explain why

hypoxia has been reported to adversely affect the healing rate of staphylococcal lesions (Ball and Schmidt, 1971). Failure to find a similar phenomenon during infection with *K. pneumoniae* indicates that specific characteristics of the bacteria must also be considered. Clearance of staphyloccoci has been reported to be inhibited by hypoxia, but the same treatment had no effect on removal of *Proteus mirabilis* from mouse lungs (Schmidt, 1969).

It can be concluded that artificially induced hypoxia, of sufficient degree begun before infection is well established, can prolong survival times of mice infected with *S. aureus* or *K. pneumoniae*, apparently through effects on host metabolism.

## SUMMARY

Comparative studies of the effect of hypoxic stress on infection with gram positive or gram negative bacteria were conducted. Survival times of mice infected with either *S. aureus* or *K. pneumoniae* were significantly extended by treatment with 10% oxygen begun immediately post infection. Delayed treatment was ineffective. Hypoxia induced by 0.6 atm. was detrimental in treatment of infected mice. This latter treatment had no effect on bacterial counts in organs of infected mice. Treatment with 10% oxygen did, however, increase numbers of *S. aureus* in lungs and livers of infected mice. No effect of this treatment on bacterial counts was seen in mice challenged with *K. pneumoniae*. Mice with diffuse type haemoglobin were found to be more resistant to both infections than animals with the single type haemoglobin. Thus, under some circumstances, hypoxia can prolong survival times of mice infected with *S. aureus* or *K. pneumoniae*, apparently through effects on host metabolism.

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